

Serial No.: 09/898,324

IN THE CLAIMS:

1. (Currently amended) A method for sequence-specific identification, separation and quantitation of ~~polynucleotide~~ a subset of restriction fragments in a population of ~~polynucleotides~~ restriction fragments, the method comprising:

- (a) reverse transcribing an RNA population to provide a double-stranded cDNA population;
- (b) digesting said cDNA population with one or more restriction endonucleases having a degenerate recognition or cleavage sequence, wherein said restriction endonuclease is a three- to eight-base cutter and wherein the degenerate recognition ~~[[of]]~~ or cleavage sequence is represented by the formula N^m , where N is the extent of degeneracy, and m is the number of degenerate bases, and wherein for at least one of said restriction endonucleases N is 2-4 and m is 1-5, to produce restriction fragments having N^m different single-stranded overhangs for each restriction endonuclease;
- (c) ligating said restriction fragments to a series of adapters lacking restriction endonuclease sites, each adapter having a sequence complementary to one of said overhangs such that restriction fragments having identical overhangs are ligated to the same adapter, wherein each ligating reaction is performed with one adapter of said series of adapters and said one adapter can be ligated to only a subset of said restriction fragments;
- (d) amplifying said subset of said restriction fragments for no more than 25 cycles with a primer comprising a detectable label, wherein said primer is designed to amplify only those restriction fragments to which said one adapter of said series of adapters has been ligated; and
- (e) detecting and quantifying said subset of restriction ~~polynucleotide~~ fragments.

2. (Original) The method of claim 1 wherein for at least one of said restriction endonucleases m is 2, 3, or 4.

Serial No.: 09/896,324

3. (Original) The method of claim 1 wherein said restriction endonuclease comprises a four-base cutter.

4. (Original) The method of claim 1 further comprising digesting the restriction fragments obtained in (b) with one or more further restriction endonucleases producing restriction fragments with single-stranded overhangs different from those produced in (b).

5. (Currently amended) The method of claim 4 further comprising ligating the single-stranded overhangs produced by the digesting of claim 4 to a series of adapters, each adapter having a sequence[[s]] complementary to one of said overhangs.

6. (Original) The method of claim 1 wherein said restriction fragments of (d) are amplified by the polymerase chain reaction (PCR) to produce PCR products.

7. (Original) The method of claim 6 wherein said adapters provide priming sites for said polymerase chain reaction.

8. (Currently amended) The method of claim 6 further comprising detecting and quantifying the PCR products.

9-23. (Cancelled)